Understanding the Onset of Health Impacts Caused by Disturbances

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LONG-TERM GOALS

Here we propose to make use of the recent advances in transcriptomics and metabolomics to define adequate ecological health indicators for marine mammals. We distinguish such measures from currently used health indicators as primarily focusing in defining the capacity of an individual to contribute, through survival and reproduction, to its population. We aim to develop these health ecological indicators that can be related to both changes in activity caused by disturbances as well as individual's demographic contributions. These indicators will define the PCoD Health stage in a way that we can start to integrate ecological and physiological PCoD research.

OBJECTIVES

In order to understand the onset of physiological impacts, we need to understand how the link between disturbance exposure and physiological changes are internally triggered by changes in gene expression and propagated to the physiology through changes in metabolism. These changes can be detected by estimating metabolomic changes in a range of tissues for the affected individuals (Unternaehrer et al. 2012). There is an active development of the understanding of the transcriptomic and metabolomic foundations of marine mammal physiology (e.g., Boaz et al. 2012; Champagne et al. 2013). Unfortunately, we do not have marine mammal dataset detailed enough to address this question. To define demographically relevant indicators in marine mammals, we need to assess how the fat stores-hypothalamus axis changes in response to disturbance such as foraging disruption. To do so, we will use a controlled experiment on a model species. In this setting we will be able to continuously monitor activity as well as obtain repeated estimates of health and metabolism. In addition, we can obtain circulating hormonal and transcriptomic and metabolomic composition of key tissues at the end of the experiment. We will infer the major biological pathways linked to changes in activity potentially caused by the disturbance that can also be linked to changes in health and condition. We can then assess longitudinally the variability we observed in the expression of these biological pathways in individual bottlenose dolphins with known health status. This will increase the resolution in linking health to demographic contributions of individuals. We will for the first time assess the relevance of adipose transcriptomic and metabolomic biomarkers as measures relevant to PCoD in cetaceans.
We aim to meet the following objectives:

- Infer continuous changes in health in a uniquely rich and large dataset of mice exposed to graded levels of foraging disruptions

- Determine how changes in activity in this experimental system are related to changes in the health of individuals

- Assess the biological pathways that are changed during health degradation in the adipose and hypothalamus tissues

- Determine which of those pathways are ecologically relevant

- Assess changes in the metabolomic interaction network in bottlenose dolphins from longitudinal studies and targeted challenges on individuals using pre-existing data.

- Use this network to estimate the biological pathways detected in the mouse model

- Determine the variability in those pathways among individuals of the same populations which had different health status and before and after challenges for the same individuals.

APPROACH

The Population Consequences of Disturbances (PCoD) paradigm provides a mean to link perturbations of individual phenotypic dynamics (encompassing both activity and physiological characteristics) to demography. A crucial process in PCoD is to define a measure of health that can mediate behavioral perturbations to the demographic contributions of individuals. We currently use coarse health indicators such as a measure of body condition based on visual assessment, or on estimated mass. The relationship between those and individual survival and reproductive success is very noisy. Other health metrics are available from physiological and veterinarian marine mammal studies (Reif et al. 2008). We can use those to distinguish between individuals in good and poor health. We can also use those to assess the magnitude of the physiological response of an individual to stressful events (Fair et al. 2014). However, we are less able to determine the demographic contributions an individual can make depending on its health status defined in those manner. There is therefore a need to develop integrative health metrics that can capture both the multiple dimensions defining individual health and the influence health has on demographic contributions; we need ecological health indicators.

Both physiological and ecological studies point to fat stores, such as blubber, as a tissue playing an active role in linking cetacean behavior to demography (e.g., Huang et al. 2011). More broadly, in mammalian studies, individuals placed under foraging constraints will also modulate their fat stores (e.g., Hambly and Speakman 2005). Changes in fat stores will trigger signals, including leptin and insulin, which will play a regulatory role in activity budget and energy partitioning and therefore demographic contributions. Many of these signals can be detected by the expression of the genes and metabolites involved in their biological pathways. We are only now starting to understand the diversity of pathways through which the adipose can affect these changes (Lafontan 2008).

To do so, we will focus on interactions between adipose and hypothalamus biological pathways and relate changes in these tissues to current health indicators – integrating energetic, hormonal, metabolomic and transcriptomic measures to determine the health of individuals. We will make use of a uniquely rich experiment on mice (Mitchell et al. 2015a) to detect changes in biological pathways in the adipose and hypothalamus when they face foraging disruptions similar to the disruptions observed through acoustic perturbations of cetaceans. This study will identify the biological pathways that are relevant ecological health indicators. We will then use two unique set of bottlenose dolphin longitudinal studies to assess the variability in these biological pathways, using plasma metabolome, among individuals of varying health and condition. This approach will provide a mechanism to develop biomarkers for PCoD that are easier to interpret biologically and also more robust to physiological variability that might exist within- and between- species.

WORK COMPLETED

Contract was finalised on 24 August 2015. The PDRA post is now advertised. Current regulations require us to advertise this post internally for four weeks. If no successful candidate is selected at that stage, the post will be advertised externally for 30 days starting 12 October 2015. We anticipate the PDRA to be in place in January 2016.

RESULTS

Parallel work on the mouse model study is providing a clear framework to commence investigating health changes associated with the phenotypic changes caused by disturbances.

Calorie restriction led to a reduction in body mass, but this reduction emerged from differential weight loss across organs with white adipose tissue (WAT) being preferentially used under calorie restriction (Figure 1, Mitchell et al. 2015a). This gradual body mass loss in relation to graded calorie restriction exposure was matched by a gradual decrease in reproductive investment (Figure 2, Mitchell et al. 2015b). However, it was not matched by a graded change in behavioural phenotype (Lusseau et al. 2015). Behavioural phenotyping showed that the mice activity characteristics and dynamics changed substaintially only for high levels of calorie restriction. Also, changes propagated throughout the activity repertoire of mice and was not limited to foraging activities. We see an increased variabilityin behavioural response for intermediate level of calorie restriction (Lusseau et al. 2015) which will be crucial to relate to variability in health metrics.
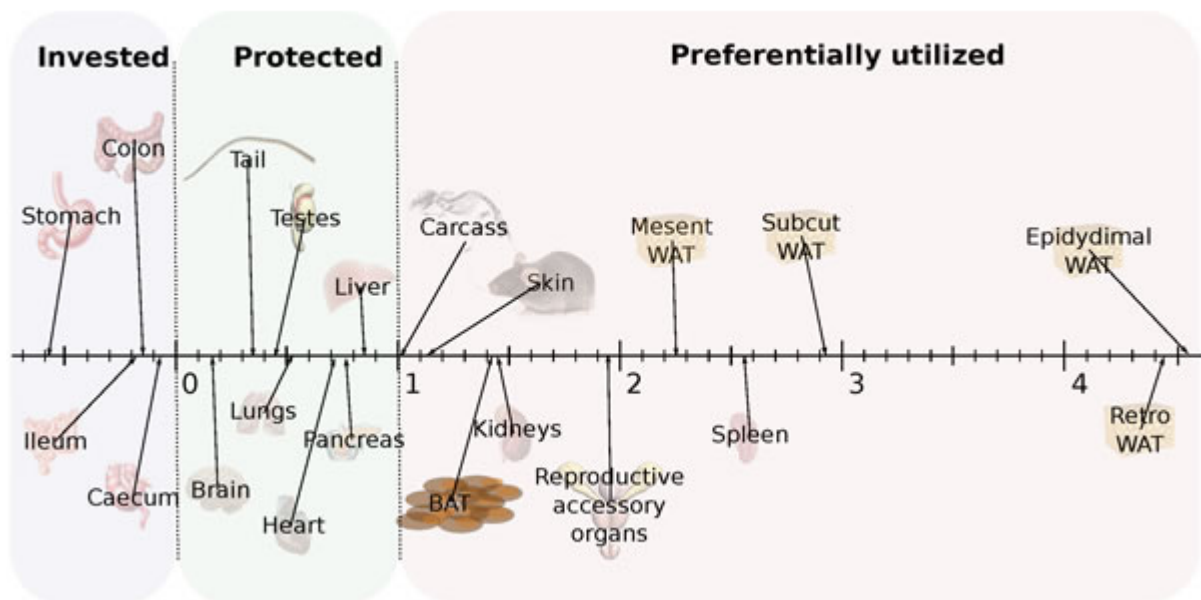
Figre 1. Variability in the way organ mass changes under calorie restriction (decreased feeding). Adapted from Mitchell et al. 2015a: The x-axis represents the relationhip between the log organ mass and the log body mass after 3 months of calorie restriction in mice.

We have now identified differential gene expression related to calorie restriction in the hypothalamus of the same mice (Derous et al. to be submitted) and differential gene expression analysis in WAT is ongoing. This provides the foundation to identify the biological pathways changed by foraging disruption.
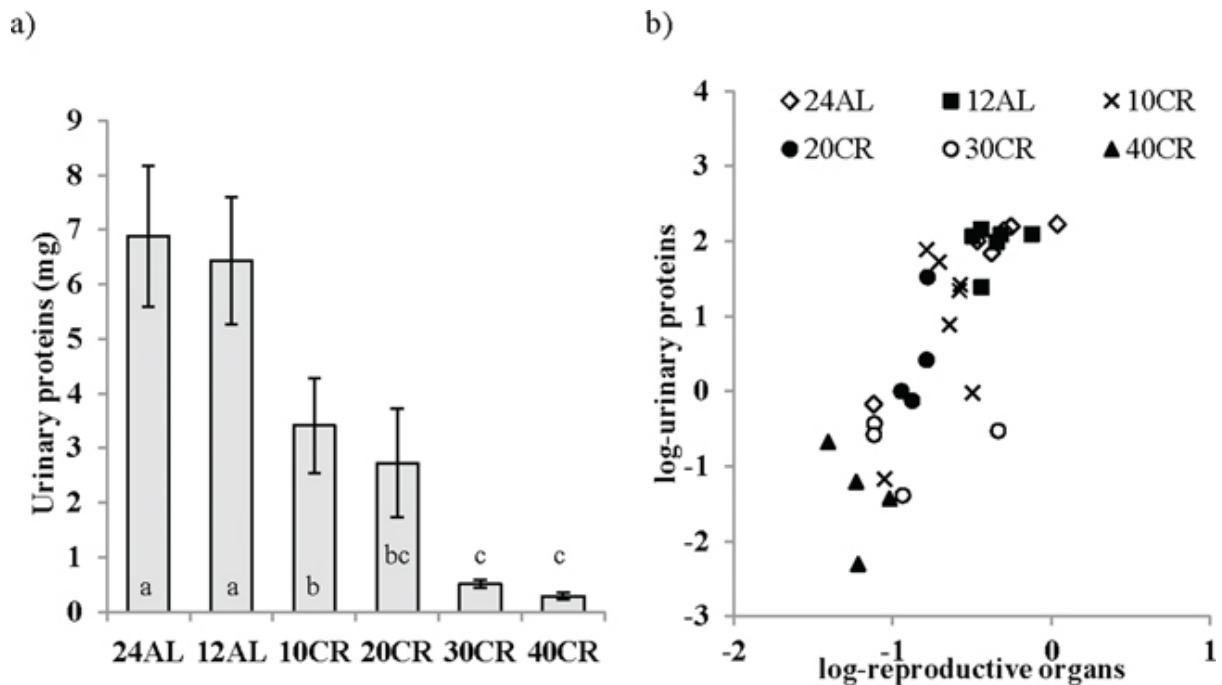
Figure 2. Mice exposed to 3-month calorie restriction reduce their reproductive investment (adapted from Mitchell et al. 2015b). The level of major urinary proteins (MUPs), a proxy for reproductive investment in male mice, a. lowered after calorie restriction when contrasting mice that had ad-libitum access to food for 24 hours (24AL) or 12 hours (12AL) to mice that had diets with 10-40% calorie restriction (10CR to 40CR), and b. was related to a disinvestment in reproductive organs (reduction in organ mass).

The delay in contracting is causing a change in project scheduling. There is no impact on ability to carry out the work, but this will impact timeline of milestone:

	Sept 2015					Feb 2017
	Q1	Q2	Q3	Q4	Q5	Q6
Determine changes in the biological pathways in the adipose-hypothalamus axis in mice	→————→					
Develop interaction network inference method	————————→					
Determine changes in challenged *Tursiops* metabolome network			————————→			
Determine variability in *Tursiops* metabolome network depending on health status			———————→			
Determine changes in biological pathways identified in the mouse model from *Tursiops* metabolome network					————————→	
Reporting and submission for publication	→			→		→

IMPACT/APPLICATIONS

We will address needs to assess population consequences of acoustic disturbance (PCAD using PCOD framework) and provide a conceptual framework to inform environmental compliance efforts. The goal of this project is complementary to the work of the current EOS-Stress and EOS-

PCAD working groups. It represents the first effort to develop a conceptual framework to integrate information emerging from both working groups and increase the resolution with which we can determine health impacts emerging from disturbances, considering both pathways concurrently.

RELATED PROJECTS

None

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